

Principle of introduction of Fluo3 into cells and Ca²+ measurement

B cell recaptor

Excitation

Fluo3/AM

Fluo3/AM

Fluo3/AM

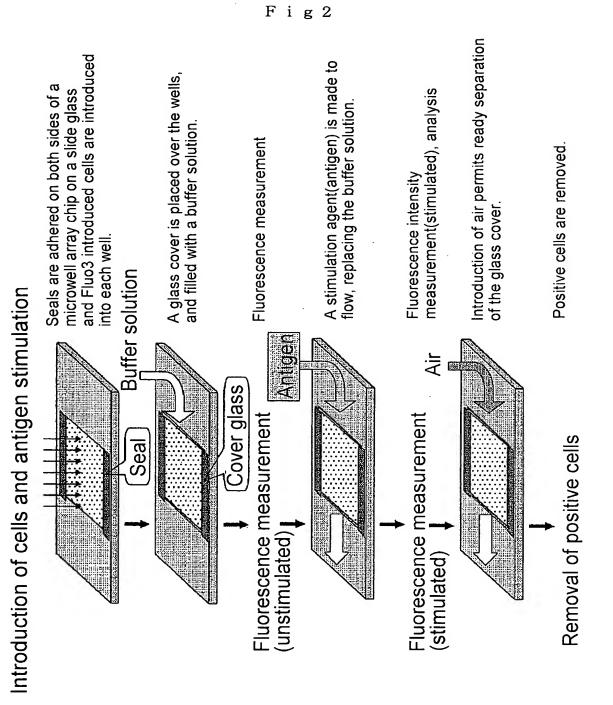
Fluosom dependent

fluorescent dye)

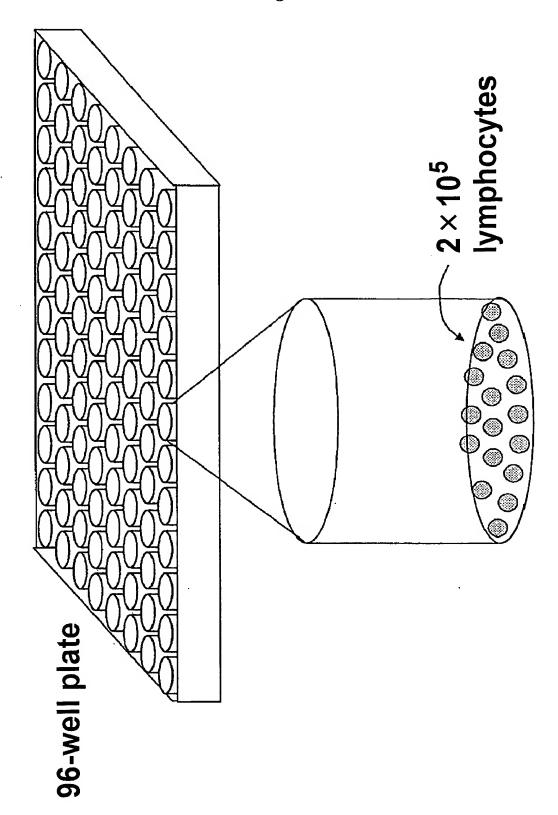
Fluorescence

Antigen

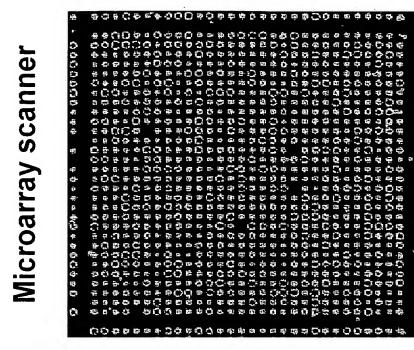
Fluo3/AM is digested with an enzyme in a cell to form Fluo3, Which binds to Ca²⁺, When an antigen binds to a B-cell receptor, Ca2+ concentration in a cell increases. resulting in generation of fluorescence.



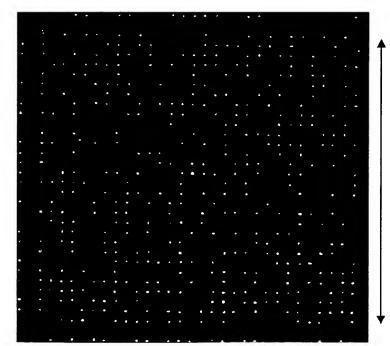
F i g 3



F i g 4

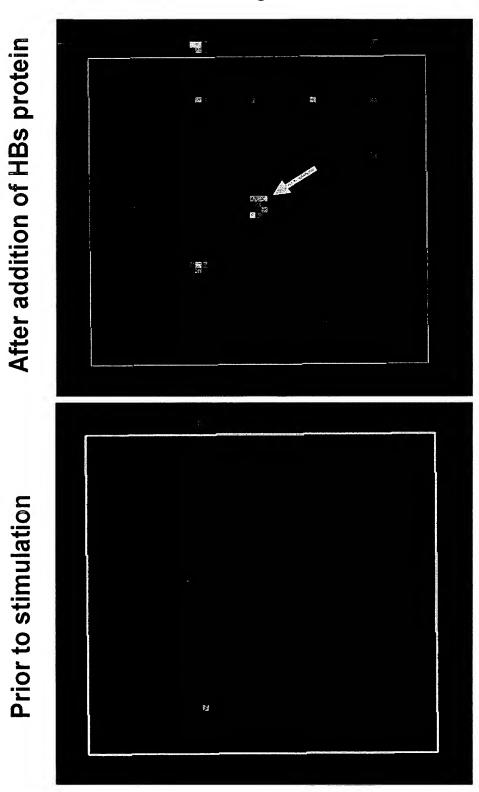


Fluorescence microscopy



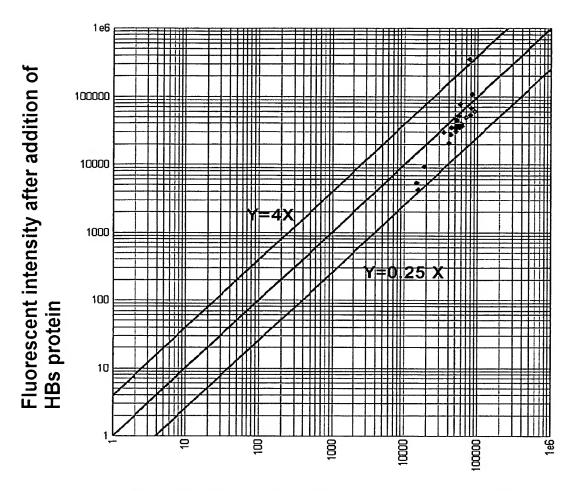
One cluster = 30×30 wells

F i g 5



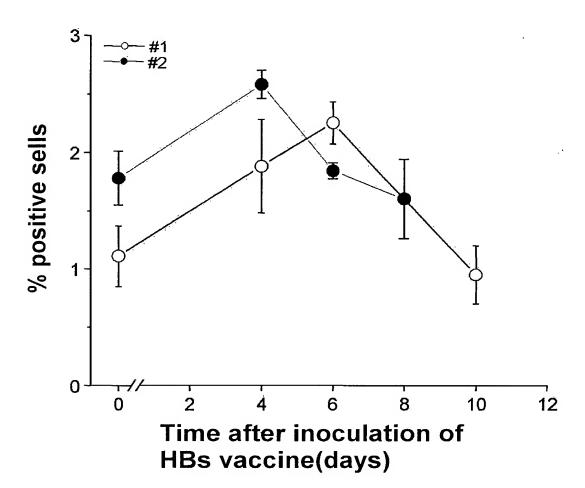
Best Available Copy

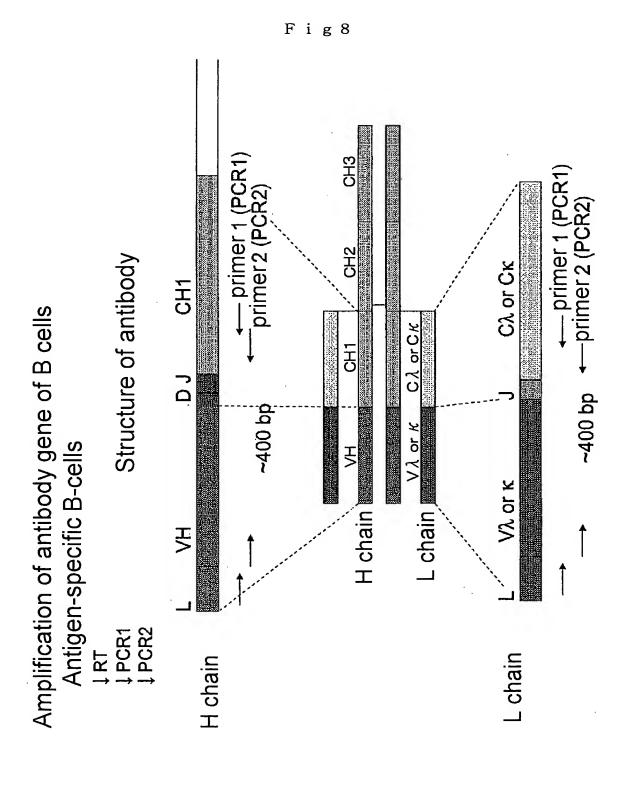




Fluorescent intensity prior to stimulation







1 st	H	cagtotocagocacootgtotttgtotocaggggcaaagtagccacoototoctgcnggg 60
2nd:	55	cagtotocagocacoctgtotttgtotocagggg-aaag-agocacoctotoctgcaggg 112
1st:	61	ccagtcagagtgttagcanctacttagcctggtaccaacagaaacactggccaggctccc 120
2nd:	11.3	ccagtcagagtgttagcagctacttagcctggtaccaacagaaac-ctggccaggctccc 171
1st:	121	aggetectnatetatgatgeateteaacagggeeactggeateecageeaggttaagtgg 180
2nd:	172	aggetecteatetatgatgeate-caacagggeeactggeateecageeacetteagtgg 230
1st:	181	cagtgggtctgggacagacttcactctcaccatcancagcctagagcctgaagattntgc 240
2nd:	231	cagtgggtctgggacagacttcactctcaccatcagcagcctagagcctgaagattttgc 290
1st:	241	agttnattactgtcancagcgtatcaactggcctctcactttcggcggagggaccaaggc 300
2nd:	291	agtttattactgtcagcagcgtagcaactgggtgctcactttcggcggagggaccaagg- 349

tggagatcaaacgaactgtggctgcaccatctgtc 335

2nd: 350 tggagatcaaacgaactgtggctgcaccatctgtc 384

tgtgcgagacag 357

2nd: 346

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2nd:	4 8	gtcccagctgcagctgcagg-agtcgggcccag-gactggtgaagccttcggag 10	Ŕ
1st:	61	accetgteceteacetgeactgtetetggtggetecateageagtagtagttaetaetgg 120	0
2nd:	106		S.
18t:	121	ggctggatccgccagccccagggaaggggctggagtggattgggagtatctattatagt 180	0
2nd:	166	ggctggatccgccagccccagggaaggggctggagtggattgggagtatctattatagt 225	2
1st:	181	acctactacaa	0
2nd:	226		ю
1st:	241	sagtteteeetgaagetgagetetgtgaeeggeegeagaeaeggetgtgtattae 3	0
2nd:	286		10
lst:	301	tgtgcgagacag 312 	
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